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# Mycopathologia

## Subcutaneous mycotic cyst caused by *Rousoella percutanea* in a UK renal transplant patient.

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Abstract:	<p>Fungi from more than one hundred genera have been implicated in subcutaneous fungal infections, usually following traumatic inoculation of the etiologic agent. With the advent of molecular approaches to fungal identification and taxonomy, novel agents of subcutaneous mycoses are increasingly reported. In this manner, <i>Rousoella percutanea</i>, a novel species in Pleosporales, was described in 2014 from a subcutaneous mass in an immunocompetent male adult. A second case was discovered after analysis of historical culture collection isolates, from a pedal mass in a renal transplant patient. Here we describe a new case of subcutaneous <i>R. percutanea</i> infection, causing a mycotic cyst in a renal transplant patient. Although fungal infection was confirmed histologically, viable fungal isolates could not be recovered in culture from biopsy material and identification of the causative agent relied upon PCR amplification and sequencing of fungal rDNA genes. This is only the third reported case of human infection with <i>R. percutanea</i> worldwide, the second in a renal transplant patient, and the first from a patient resident in the UK. The current case illustrates the importance of molecular approaches for the identification of emerging fungal pathogens in culture-negative subcutaneous fungal infections.</p>
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# Subcutaneous mycotic cyst caused by *Roussoella percutanea* in a UK renal transplant patient.

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Running Title: *Roussoella percutanea* mycotic cyst

Key words: *Roussoella percutanea* / emerging pathogen / rDNA sequencing / subcutaneous infection / renal transplant/ *Pleosporales*

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## ABSTRACT

Fungi from more than one hundred genera have been implicated in subcutaneous fungal infections, usually following traumatic inoculation of the etiologic agent. With the advent of molecular approaches to fungal identification and taxonomy, novel agents of subcutaneous mycoses are increasingly reported. In this manner, *Roussoella percutanea*, a novel species in *Pleosporales*, was described in 2014 from a subcutaneous mass in an immunocompetent male adult. A second case was discovered after analysis of historical culture collection isolates, from a pedal mass in a renal transplant patient. Here we describe a new case of subcutaneous *R. percutanea* infection, causing a mycotic cyst in a renal transplant patient. Although fungal infection was confirmed histologically, viable fungal isolates could not be recovered in culture from biopsy material and identification of the causative agent relied upon PCR amplification and sequencing of fungal rDNA genes. This is only the third reported case of human infection with *R. percutanea* worldwide, the second in a renal transplant patient, and the first from a patient resident in the UK. The current case illustrates the importance of molecular approaches for the identification of emerging fungal pathogens in culture-negative subcutaneous fungal infections.

191 words

## 47 Introduction

48 Subcutaneous fungal infections usually occur following traumatic inoculation of the  
49 etiological agent into cutaneous or subcutaneous tissue. Although infections typically remain  
50 localised in immunocompetent individuals, without treatment they frequently become  
51 chronic, with gradual expansion to involve adjacent tissues and even bone (1-3). This  
52 heterogeneous disease spectrum includes hyalohyphomycoses (infection by fungi with  
53 hyaline hyphae), phaeohyphomycoses (infection with melanised, dematiaceous fungi),  
54 chromoblastomycoses (characterised by formation of sclerotic bodies in tissue) and  
55 eumycetoma (characterised by extensive tumefaction with formation of purpurulent  
56 sinuses extruding fungal grains) (2-5).

57 Fungi from more than 100 genera have been reported from subcutaneous mycoses in  
58 humans, with novel species and genera described almost weekly (5). In some cases, the  
59 responsible fungi have only been reported from individual, isolated case reports suggesting  
60 that they are infrequent agents of subcutaneous infection (6-9). For many other species, they  
61 have been predominantly associated specifically with precise clinical presentations and have  
62 been rarely or never isolated from nature (2, 4, 5, 10-12). Although certain species are  
63 predominantly associated with a specific clinical presentation, clinical picture is also  
64 determined by host immune status, with the result that accurate definition of disease entity  
65 may be difficult (13, 14). In addition, many of the agents of subcutaneous mycoses exhibit  
66 reluctant conidiation in culture, especially when isolated from chronic cases (5, 10, 12, 14).  
67 Since accurate identification of the causative agent is paramount in directing appropriate  
68 therapeutic interventions, molecular approaches using PCR amplification and sequencing of  
69 conserved regions of fungal genomic DNA is increasingly employed (14-17). Using such  
70 approaches, *Roussioella percutanea*, a novel species in *Pleiosporales*, was recently described

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71 from a subcutaneous mass in an immunocompetent male adult (18). Clinical presentation  
72 included hyphal masses with granulation tissue and fibrosis, but draining sinuses typical of  
73 eumycetoma were absent. A retrospective analysis of other culture collection isolates  
74 revealed one further historical isolate of *R. percutanea*, from a pedal mass in a renal  
75 transplant patient who also presented with necrosis and inflammation but without draining  
76 sinuses or fungal grains (13,18). In both cases, conidiation in culture was delayed or absent,  
77 and variable antifungal susceptibility profiles were reported for the two previous isolates  
78 (18).

79 Here we present the first case of *R. percutanea* infection from the UK, involving a  
80 subcutaneous cyst from a renal transplant patient. Attempts to culture the organism from cyst  
81 tissue were unsuccessful and diagnosis and identification were achieved by PCR  
82 amplification and sequencing of fungal genomic DNA from biopsy material.

## 83 84 **Case History**

85 A 47 year old male, originally from Surat, Gujarat, but residing in the UK since 1997,  
86 underwent a renal transplant in November 2015 for end-stage renal failure of unknown cause.  
87 Post-operatively he was immune-suppressed with oral prednisolone 12.5mg once a day and  
88 tacrolimus 5mg twice daily and commenced prophylactic oral co-trimoxazole and aciclovir.  
89 Other past medical history included gout, osteoarthritis, an inguinal hernia and migraines.  
90 Post-transplant he suffered from an episode of declining renal function secondary to  
91 borderline T-cell mediated rejection, requiring two rounds of pulsed methylprednisolone and  
92 addition of mycophenylate mofetil 500mg twice daily to his immunosuppressive regimen  
93 from January 2016. His clinical course was also complicated by multiple episodes of  
94 urosepsis, including one episode of bacteraemia, due to an extended-spectrum  $\beta$ -lactamase  
95 producing *Klebsiella pneumoniae*, between January and May 2016.

During admission for an episode of *Klebsiella* urosepsis in March 2016 an incidental finding was made of a mobile, soft tissue mass over the right Achilles tendon. The lesion was non-tender and not adherent to the tendon itself with no masses elsewhere or evidence of lymphatic involvement or sinus tract formation. The patient had been unaware of the lesion and it had not caused him any pain or symptoms. Clinical suspicion of mycetoma was raised. Further travel history included trips to Dubai 2005 and Morocco in 2014. The patient could not re-call any specific injuries or possible inoculation events.

An ultra-sound scan confirmed a 2 by 1.5 centimetre heterogeneous nodule overlying but separate from the Achilles tendon. A biopsy of the lesion was performed which did not reveal any organisms on Gram stain and there was no growth by conventional bacterial culture (including Sabouraud's dextrose agar). Histology revealed a diffuse macrophage infiltrate with cytoplasmic inclusions and fungal hyphae were seen on Periodic acid-Schiff and Periodic acid-Schiff-diastase stains. Empirical antifungal treatment was not started at this point due to the patient's stable clinical condition.

Microscopic analysis of biopsy material by potassium hydroxide digestion with Calcofluor enhancer (Bactidrop, Remel) revealed the presence of moderate amounts of filamentous fungal elements. These were generally amorphous, and included fine, regularly septate and acutely branching hyphae, which were hyaline to light brown together with more common short chains of swollen cells and chlamydospores. Despite prolonged culture on a variety of mycological media, no viable fungal isolate could be recovered for further analyses. Small sections of biopsy tissue were subjected to digestion and Qiagen column purification (Qiagen Mini Blood kit) and PCR amplification of the 28S rDNA and Internal transcribed spacer 1 (ITS1) regions of the resulting extracted fungal genomic DNA using the PCR primers and conditions described previously (12, 14, 17). The sequences of the resulting PCR amplicons were 100% identical to those of the type species of *Roussoella percutanea*



(isolate CBS128203; GenBank accession numbers KF366448 and KF322117) present in the public synchronised databases. Treatment with oral voriconazole 200mg twice a day was then immediately commenced and total excision of the lesion took place with 5mm margins. Histology of the excised lesions confirmed fungal hyphae on both Hematoxylin/Eosin and Grocott stains. Unfortunately, due to clinical concern regarding potential interactions between voriconazole and tacrolimus, and a falling neutrophil count (to a trough of  $2.1 \times 10^9$ /litre) whilst being treated with voriconazole, anti-fungal treatment was discontinued after only 2 weeks. Despite this, the patient's wound is healing and dry at 6 weeks post-excision with no evidence of recurrence so far.

130

## Discussion

We report here the first known UK case of subcutaneous infection by *R. percutanea*. To our knowledge, this is only the third case reported worldwide, and the second to have affected a renal transplant patient (13,18). In all three cases, presentation differed from that seen with eumycetoma due to the presence only of inflammatory infiltrates and discrete fungal elements and absence of fungal grains or draining sinuses. Subcutaneous mycoses in immunocompetent patients frequently present diagnostic challenges due to the heterogeneous nature of clinical presentations and the fact that clinically detectable infections can take years to develop. This situation is further compounded in transplant patients, where relatively dormant fungi that have been harboured subcutaneously for many years or decades become clinically active after immunosuppression. Although most subcutaneous mycoses are thought to result from traumatic inoculation, it is hardly surprising given this protracted and variable incubation period that most patients are unable to recall any history of injury at the affected sites. This was the case with the current patient and with the two previous reports of *R. percutanea* subcutaneous mycoses published to date (13, 18).

Previous reports have suggested strain-specific variations in antifungal susceptibility in *R. percutanea* (18), indicating that specific testing of patient isolates should be used to guide antifungal therapeutic decisions. Since the current case was culture-recalcitrant and no isolate was available for testing, the patient was treated with total excision of the lesion and commenced on voriconazole therapy. Unfortunately, antifungal therapy was stopped after only 2 weeks due to drug interactions. Although the patient is currently well, close follow up will be required, especially in the light of the reports of recurrences in both previous cases of *R. percutanea* (13,18). Finally, the current case further highlights the utility of molecular diagnostic and identification approaches in medical mycology, especially in cases of subcutaneous fungal infections where conventional identification approaches are hindered by

difficulties in recovering viable fungi from biopsy samples and organisms that fail to produce distinctive features in culture.

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#### Figure Legend.

Figure 1. Histopathological appearance of biopsy material excised from the subcutaneous cyst. H&E stain. Panel B is a magnification of a section of panel A. Scale Bars = 5 µm.

